## REMARKS

Claims 1-33 are pending in the application. Claim 34 was previously withdrawn from consideration. New claims 35-47 are supported by originally filed claims 2-6 and 11-18.

***Oath/Declarartion***

The Examiner has requested Applicants to provide information to correctly claim priority and submit a new oath. Applicants hereby submit a new declaration in compliance with 37 CFR §1.67(a) to claim priority to US application 09/428,350 as requested by the Examiner.

***Objection to claims 31 and 32***

Applicants have amended claim 31 and 32 to correct a typographical error and make them dependent on claim 30 instead of claim 27.

***Rejection under 35 USC §112, first paragraph***

The Examiner has rejected claims 1, 20, 25, 29, and 33 under 35 USC §112, first paragraph, for the use of the term "selectively amplifying". Claim 1 had been canceled and claims 20, 25, 29 and 33 have been amended to remove the term "selectively" and to clarify that the amplification results in the fragments that were cut by both enzymes being the predominant products in the amplified product. The amplified product is enriched for the fragments cut by both enzymes relative to the fragments cut by one enzyme.

On pages 5-7 of the office action (paper no. 10) the Examiner outlines various methods by which the fragments that are cut with one enzyme at both ends might be amplified. Applicants agree with the Examiner that amplification of the fragments that are digested on both ends with the same enzyme is possible, but respectfully disagree with the Examiner as to the extent of amplification that will result. Amplification of those fragments will be inefficient compared to the amplification of fragments that have been cut with two different enzymes because during the early cycles of amplification generation of full length product (product with both priming sites) will occur inefficiently for those fragments. The result is that the fragments cut with two different enzymes will be enriched in the amplified product relative to the fragments cut with one enzyme. The claims have been amended to reflect this. Support for the amendment may be found in the specification in Figures 1 and 4, which indicate that the fragments cut by RE1 and RE2 are the "predominant PCR product".

The fragments cut by RE1 and RE2 will be the predominant PCR products because the primers bind to the templates and extend much more rapidly and efficiently than the template strands reanneal to one another. This is a characteristic of PCR as stated in a standard manual on the subject, "Primers are present at a significantly greater concentration than the target DNA, and are shorter in length; as a result, they hybridize to their complementary sequences at an annealing rate several orders of magnitude faster than the target DNA duplex can reanneal." PCR Primer: A Laboratory Manual Dieffenback and Dveksler eds. Cold Spring Harbor Lab Press (1995). Amplification of the fragments cut with only RE1 or RE2 requires reannealing of complementary template strands or primer extension products. This is very different from the primer mediated

extension that is the object of PCR. It is well known by those of skill in the art that reannealing of the complementary strands of the template occurs very slowly and makes at most a minor contribution to the final product.

When performing PCR it is common practice among those skilled in the art to include primers at a concentration which greatly exceeds the concentration of the template. This increases the likelihood that a primer will hybridize to a template molecule. Although the fragments, which were cut on both ends by the first restriction enzyme, will go through linear amplification, the amount of amplified fragments will still be minimal compared to the existing primers in the reaction, which minimizes the subsequent amplification using the amplified fragments as primers.

Secondly, although large fragments bind tighter to their complementary fragments than (short) primers, the kinetics of hybridization of large fragments is much slower than (short) primers, which is critical in initiating amplification. It is well understood among those skilled in the art that in a PCR reaction, (short) primers rather than large fragments will preferentially hybridize and extend during amplification.

Because the fragments are in low concentration the amplification of the undesired fragments will at best require several cycles of PCR before exponential amplification is achieved. As a result the amplified sample will be enriched for the RE1/RE2 fragments relative to the RE1 or RE2 alone fragments. Because of the exponential nature of the amplification reaction even if the amplification of a fragment is delayed by a single round that results in a 50% difference in final product. Consequently, the claimed method would produce an amplified product where the predominant fragments are those that were cut by both RE1 and RE2. Accordingly, Applicants respectively request that the

Examiner reconsider and withdraw the rejection with regards to amended claims 20, 25, 29 and 33.

***Rejection under 35 USC §112, second paragraph***

In paragraph 5a. the Examiner rejected claims 1-19 as being indefinite. Applicants have canceled these claims making this rejection moot.

In paragraph 5b. the Examiner rejected claims 1, 20, 25, 29, and 33 because of the term "selectively amplifying". As discussed above claim 1 has been canceled and claims 20, 25, 29 and 33 have been amended to clarify that the fragments cut with both enzymes are the predominant PCR products.

In paragraph 5c. the Examiner rejected claims 2-7. Claims 2-7 have been canceled making this rejection moot.

In paragraph 5d. the Examiner rejected claim 11 because it was unclear how single stranded nucleic acids would be appropriate substrates for restriction enzymes. Claim 11 has been canceled making this rejection moot.

In paragraphs 5e. and 5f. the Examiner rejected claims 12-18. Claims 12-18 have been canceled making these rejections moot.

In paragraph 5g. the Examiner rejected claim 23 because of the phrase "is designed to query DNA fragments". Claim 23 has been canceled making this rejection moot.

***Rejection under 35 USC §102(b)-Smith***

The Examiner rejected claims 1-8, 10 and 11 under 35 USC §102(b) as being anticipated by Smith, PCR Methods and Applications 2:21-27, 1992 (hereinafter "Smith"). Claims 1-8, 10 and 11 have been canceled making this rejection moot.

***Obviousness rejection under 35 U.S.C. 103(a) - Smith in view of New England Biolabs, Inc. online catalog***

The Examiner rejected claim 9 under 35 USC §103(a) over Smith in view of the New England Biolabs, Inc, online catalog. Claim 9 has been canceled making this rejection moot.

***Obviousness rejection under 35 U.S.C. 103(a)- Smith in view of Makarov and Langmore***

In Paragraph 8 the Examiner rejected claims 12-19 under 35 USC §103(a) over Smith in view of Makarov and Langmore, USPN 6,197,557. Claims 12-19 have been canceled making this rejection moot.

***Obviousness rejection under 35 U.S.C. 103(a)- Smith in view of Lipshutz et al.***

In paragraph 9 the Examiner rejected claims 20-28 and 33 under 35 USC §103(a) over Smith in view of Lipshutz, et al., Bio Techniques 19(3):442-47, 1995. Smith teaches fragmenting a nucleic acid sample with two enzymes and amplifying a subset of fragments that have been cut by both enzymes. Lipshutz et al. teaches a method of discover polymorphisms using an array.

Neither Smith nor Lipshutz teach a method of reducing complexity to generate a representative subset of a large genome combined with an array designed to interrogate known polymorphisms present in that subset. Applicants' methods combine a specific complexity reduction method with an array designed to interrogate polymorphisms predicted to be in the reduced complexity sample when using the specific complexity reduction method. The complexity reduction step results in an amplified sample that is predicted to contain a known collection of fragments that carry known polymorphisms and the step of array hybridization is designed to identify the alleles present at those specific polymorphisms. In this way a representative subset of polymorphisms may be interrogated while limiting the number of probes on the array-there are no probes on the array for polymorphisms that aren't predicted to be in the reduced complexity sample or for regions that do not have a known polymorphism.

Claims 20, 25 and 33 have been amended to more clearly reflect that the method combines reduction of complexity in a predicted way with allele detection on an array specifically designed for the complexity method used. Support for the amendments may be found on page 21, lines 13-19, page 22, lines 14-17 and in original claim 23 and 24. The array is designed by predicting which fragments will be cut by both enzymes, identifying known polymorphisms on those fragments and putting probes on the array that identify which alleles of the polymorphism are present. The array of Lipshutz tiles all possible sequences in the sequence of interest so that any polymorphism at any location would be detected.

***Obviousness rejection under 35 U.S.C. 103(a)- Smith in view of Brown and Shalon***

In paragraph 10 the Examiner rejected claim 29 under 35 USC §103(a) over Smith in view of Brown and Shalon, USPN 5,807,522. Brown and Shalon also fails to teach the combination of a specific complexity reduction method to generate a representative subset of a complex nucleic acid sample with an array designed to interrogate polymorphisms present in that representative subset. For the reasons indicated above Smith fails to remedy the deficiencies of Brown and Shalon.

***Obviousness rejection under 35 U.S.C. 103(a)- Smith in view of Brown and Shalon in further view of Lipshutz et al.***

In paragraph 11 the Examiner rejected claims 30-32 under 35 USC §103(a) over Smith in view of Brown in further view of Lipshutz. Claim 30 and amended claims 31 and 32 are dependent on amended claim 29. As indicated above amended claim 29 recites a method of determining which alleles of a known polymorphism are present in a complex sample by reducing the complexity of the sample in a predictable manner so that a fragment containing the polymorphism is present in the reduced complexity sample and hybridizing the reduced complexity sample to an array designed to interrogate known polymorphisms present on fragments predicted to be in the reduced complexity sample. Brown and Shalon fail to teach the combination of a specific complexity reduction method with an array designed based on the specific complexity reduction method.

As indicated above, neither Smith nor Lipshutz et al. remedy the deficiencies of Brown and Shalon.

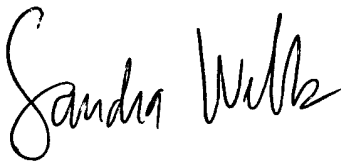
## CONCLUSION

For these reasons, Applicants believe all pending claims are now in condition for allowance and should be passed to issue. If the Examiner feels that a telephone conference would in any way expedite the prosecution of the application, please do not hesitate to call the undersigned at (408) 731-5768.

The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account 01-0431.

Respectfully submitted,

Dated: May 15, 2003



Sandra Wells

Reg. No. 52,349

Legal Department
Affymetrix, Inc.
3380 Central Expressway
Santa Clara, CA 95051
Phone: (408) 731-5768
Fax (408) 731-5392

## VERSION WITH MARKINGS TO SHOW CHANGES

*In the Specification*

On page 1, under the heading "RELATED APPLICATION", please replace the section with the following:

This is a continuation in part of [application claims the benefit of U.S.] Application No. 09/428,350 filed on October 27, 1999 [10/27/99, the entire teachings of which are incorporated herein by reference] now U.S. Patent 6,361,947, issued on March 26, 2002.

*In the Claims*

20. (Amended) A method for analyzing a nucleic acid sample comprising:

fragmenting the nucleic acid sample using a first and second restriction enzyme to produce fragments;

ligating adaptors to the fragments;

[selectively] amplifying the fragments, wherein fragments that were cut on one end by the first restriction enzyme and on the other end by the second restriction enzyme are enriched in the amplification product relative to the fragments that were cut on both ends by the same restriction enzyme;

providing a nucleic acid array consisting essentially of probes designed to detect the alleles present at polymorphisms predicted to be present on fragments that were cut on one end by the first restriction enzyme and on the other end by the second restriction enzyme;

hybridizing the amplified fragments to the array; and

analyzing a hybridization pattern resulting from the hybridization.

25. (Amended) The method of claim [21] 20 wherein the [sequence variations] polymorphisms are single nucleotide polymorphisms (SNPs).

26. (Amended) The method of claim 20 wherein [a substantial amount of the sequences predicted to be contained in the amplified fragments] polymorphisms predicted to be present on fragments that were cut on one end by the first restriction enzyme and on the other end by the second restriction enzyme are first determined by a computer system.

25. (Amended) A method of determining the alleles present at a polymorphism [screening for DNA sequence variations] in an individual comprising:

providing a nucleic acid sample from the individual;

fragmenting the nucleic acid sample using a first and second restriction enzyme to produce fragments wherein the polymorphism is predicted to be on a fragment that was cut on one end by the first restriction enzyme and on the other end by the second restriction enzyme;

ligating adaptors to the fragments; and

[selectively] amplifying the fragments, wherein fragments that were cut on one end by the first restriction enzyme and on the other end by the second restriction enzyme are predominant in the amplification product relative to the fragments that were cut on both ends by the same restriction enzyme;

providing a nucleic acid array consisting essentially of probes to determine the alleles present at polymorphisms predicted to be present on fragments that were cut on one end by the first restriction enzyme and on the other end by the second restriction enzyme;

hybridizing the amplified fragments to the array;

generating a hybridization pattern resulting from the hybridization; and

determining the alleles present at the polymorphism [presence or absence of DNA sequence variations] in the individual based upon an analysis of the hybridization pattern.

26. (Amended) The method of claim 25 wherein the [sequence variation] polymorphism is [at least one] a single nucleotide polymorphism (SNP).

29. (Amended) The method of claim 26 wherein the [at least one] SNP is associated with a disease.

30. (Amended) The method of claim 26 wherein the [at least one] SNP is associated with the efficacy of a drug.

29. (Amended) A method of determining the alleles present at a single nucleotide polymorphism [screening for DNA sequence variations] in a population of individuals comprising:

providing a first nucleic acid sample from each of the individuals;

providing a second nucleic acid sample by:

fragmenting the nucleic acid sample using a first and second restriction enzyme to produce fragments wherein the polymorphism is predicted to be on a fragment that was cut on one end by the first restriction enzyme and on the other end by the second restriction enzyme;

ligating adaptors to the fragments; and

[selectively] amplifying the fragments, wherein fragments that were cut on one end by the first restriction enzyme and on the other end by the second restriction enzyme are predominant in the amplification product relative to the fragments that were cut on both ends by the same restriction enzyme;

providing a plurality of identical nucleic acid arrays wherein the arrays consist essentially of probes to determine the alleles present at polymorphisms predicted to be present on fragments that were cut on one end by the first restriction enzyme and on the other end by the second restriction enzyme; [designed to interrogate for DNA sequence variations];

hybridizing each of the second nucleic acid samples to one of the plurality of identical arrays;

generating a plurality of hybridization patterns resulting from the hybridizations; and

analyzing the hybridization patterns to determine the alleles present at the polymorphism [presence or absence of DNA sequence variation] in the population of individuals.

30. (Amended) The method of claim 29 wherein the polymorphism [sequence variation] is [at least one] a single nucleotide polymorphism (SNP).

31. (Amended) The method of claim [27] 30 wherein the [at least one] SNP is associated with a disease.

32: (Amended) The method of claim [27] 30 wherein the [at least one] SNP is associated with the efficacy of a drug.

33. (Amended) A method of genotyping an individual comprising:

providing a first nucleic acid sample from the individual;

providing a second nucleic acid sample by:

fragmenting the nucleic acid sample using a first and second restriction enzyme to produce fragments wherein a collection of polymorphisms is predicted to be present on fragments cut on one end by the first restriction enzyme and on the other end by the second restriction enzyme;

ligating adaptors to the fragments; and

[selectively] amplifying the fragments, wherein fragments that were cut on one end by the first restriction enzyme and on the other end by the second restriction enzyme are predominant in the amplification product relative to the fragments that were cut on both ends by the same restriction enzyme;

hybridizing the second nucleic acid sample to an array designed to determine the presence or absence of one or more alleles of [a] one or more polymorphisms present in the collection of [SNPs] polymorphisms;

generating a hybridization pattern resulting from the hybridizations; and

determining the presence or absence of the one or more alleles of one or more polymorphisms present in the collection of [SNPs] polymorphisms.

35. (New) The method of claim 20 wherein the [amplified] fragments that were cut on one end by the first restriction enzyme and on the other end by the second restriction enzyme comprise at least 0.01% of the first nucleic acid sample.

36. (New) The method of claim 20 wherein the fragments that were cut on one end by the first restriction enzyme and on the other end by the second restriction enzyme comprise at least 0.5% of the first nucleic acid sample.

37. (New) The method of claim 20 wherein the fragments that were cut on one end by the first restriction enzyme and on the other end by the second restriction enzyme comprise at least 3% of the first nucleic acid sample.

38. (New) The method of claim 20 wherein the fragments that were cut on one end by the first restriction enzyme and on the other end by the second restriction enzyme comprise at least 12% of the first nucleic acid sample.

39. (New) The method of claim 20 wherein the fragments that were cut on one end by the first restriction enzyme and on the other end by the second restriction enzyme comprise at least 30% of the first nucleic acid sample.

40. (New) The method of claim 20 wherein the nucleic acid sample is genomic DNA, DNA, or double stranded cDNA derived from RNA, total RNA or mRNA.

41. (New) The method of claim 20 wherein ligation of one strand of each adaptor to the fragments is blocked.

42. (New) The method of claim 41 wherein ligation is blocked by introducing a gap of at least one nucleotide between one strand of the adaptor and one strand of the fragment.

43. (New) The method of claim 41 wherein ligation is blocked by the absence of a phosphate at the 5' end of an adaptor strand.

44. (New) The method of claim 41 wherein ligation is blocked by the presence of a modified nucleotide at the 5' or 3' end of an adaptor strand.

45. (New) The method of claim 41 wherein ligation is blocked by a terminal modification in one strand of an adaptor.

46. (New) The method of claim 41 wherein ligation is blocked at the 5' end of one strand of one adaptor and at the 3' end of one strand of the other adaptor.

47. (New) The method of claim 41 wherein ligation is blocked at the 5' end of both strands of one adaptor and at the 3' end of both strands of the other adaptor.